Studies on the nature of the pathogenicity of soil-borne *Cylindrocarpon* species

by

Mark William
M.W. Sweetingham, B.Agr.Sc.(Hons.)

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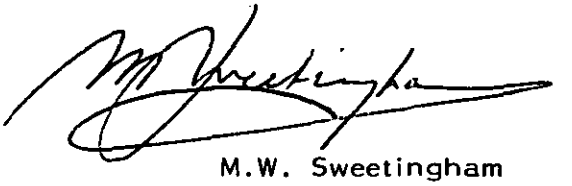
This thesis contains no material which has been accepted for any other degree or diploma in any university, and to the best of my knowledge, contains no copy or paraphrase of material previously published or written by any other person, except where due reference is made in the text of the thesis.



M.W. Sweetingham

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SUMMARY

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A study was made of the association of Cylindrocarpon species with the below-ground portions of a range of plants in Tasmania, and of the pathogenic effects of members of the genus.

A selective medium was developed to enhance the recovery of Cylindrocarpon species from soil and plant tissue. Several species were isolated from the root surfaces of a range of apparently healthy woody and herbaceous plants. Cylindrocarpon species were also isolated from the discoloured vascular tissue in the basal stem portion of several diseased woody plant species. C. destructans was the most frequently isolated species, but C. didymum, C. obtusisporum, C. tenue, C. theobromicola, C. candidum and an undescribed species were also recorded. The cultural characteristics and morphology of the isolates were described.

Most of the Cylindrocarpon isolates produced either the metabolite brefeldin A or radicicol when cultured on Czapek Dox medium. The non host-specific phytotoxicity of brefeldin A was confirmed and the spectrum of its antifungal activity was also examined.

A brefeldin A producing strain of C. destructans was consistently isolated from the discoloured wood in the base of the trunks of mature grapevines suffering from a dieback disease. The xylem vessels and functional phloem became blocked with tyloses and gum and fungal hyphae were visible in the ray cells of the diseased tissue. No other potential pathogens were isolated from the diseased tissue. Field observations suggested that the waterlogging of the trunks at ground-

level was necessary for the disease to develop. A waterlogging stress was required for the fungus to reproduce the characteristic disease symptoms on inoculated six-month-old vine cuttings. However, brefeldin A was not detected in naturally diseased grapevine tissue and could not be demonstrated to have a role in this disorder.

Certain brefeldin A producing strains of Cylindrocarpon were capable of parasitizing the root cortex of radiata pine seedlings under sterile conditions, causing a suppression in root growth. Radiata pine seed germinated in the presence of brefeldin A similarly produced stunted roots. Likewise, the growth of strawberry plants was significantly reduced, in the absence of other disease symptoms, when grown in sterilised soil amended with a brefeldin A producing isolate of C. destructans. This effect was reproduced by growing strawberry plants in soil amended with brefeldin A. Brefeldin A was absorbed by the strawberry roots and translocated into the shoots.

All the Cylindrocarpon isolates were shown to produce a spectrum of pectin degrading isoenzymes when cultured on appropriate media. Inoculation experiments showed several Cylindrocarpon species were capable of producing "soft rot" type lesions on carrot roots. Several species could also infect the hypocotyl of radiata pine seedlings in sterile media, causing post emergence damping-off. Pectin lyase enzymes were detected in the lesions from both carrot roots and radiata pine hypocotyls and may be responsible for the tissue maceration. However, virulence of the isolates was not related to pectin lyase production in vitro.

ABBREVIATIONS USED THROUGHOUT THE TEXT

CHEMICALS AND MEDIA:

Tris:	tris(hydroxymethyl)aminomethane
PVP(40):	polyvinylpolypyrrolidone (Average molecular weight: 40,000)
PDA:	potato dextrose agar
CDA:	Czapek-Dox agar

UNITS:

m:	metre	kg:	kilogram
cm:	centimetre	g:	gram
mm:	millimetre	mg:	milligram
μ:	micron ($=10^{-6}$ metre)	μg:	microgram
nm:	nanometer	ng:	nanogram
		M:	Molar concentration (moles per litre)
L:	litre	ppm:	parts per million (concentration)
ml:	millilitre	s:	second
μl:	microlitre	°C:	degrees Centigrade
		μE:	microeinstein
		Amp f.s.d.:	Ampere (full scale deflection)
		kV:	kilovolt
		eV:	electron volt

STATISTICS

S.E. of mean:	standard error of the mean
LSD (p = 0.05):	least significant difference at the 5% probability level
LSD (p = 0.01):	least significant difference at the 1% probability level